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The use of sodium carbonate to improve curing treatments against green and blue moulds on citrus fruits

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Abstract: The effectiveness of curing oranges and lemons at 33 °C for 65 h followed by storage under ambient and cold-storage conditions was investigated. This treatment effectively reduced the incidence of *Penicillium digitatum* (Pers) Sacc and *P italicum* Wehmer decay on inoculated and naturally infected oranges and lemons stored at 20 °C for 7 days. However, it failed to control green and blue mould infections on fruits placed in long-term cold storage, except green mould on oranges, which was effectively controlled. Dipping fruits in a sodium carbonate solution (20 g litre^{-1}) for 2.5 min following a curing treatment at 33 °C for 65 h satisfactorily reduced green and blue mould incidence during subsequent long-term storage at 4 °C on oranges and at 10 °C on lemons. The efficacy was greater on injured fruits inoculated after the combination of treatments was applied, achieving a 60–80% reduction in decay in comparison with the curing treatment alone in all cases. A significant reduction of blue mould was also observed on fruits inoculated both before the treatments and on those re-inoculated after the treatments, demonstrating both protectant and eradicant activity. Thus, combining curing at 33 °C for 65 h with sodium carbonate treatment effectively controlled these post-harvest diseases on artificially inoculated citrus fruits and protected against re-infection. With naturally inoculated lemons, curing followed by sodium carbonate significantly reduced both green and blue mould incidence, but was not superior to curing alone. With naturally infected oranges, curing significantly reduced blue mould, but decay was not reduced further when followed by sodium carbonate treatment.

Keywords: *Penicillium digitatum*; *Penicillium italicum*; post-harvest heat treatments; soda ash; long-term storage

1 INTRODUCTION

Post-harvest green mould, caused by *Penicillium digitatum* (Pers:Fr) Sacc, and post-harvest blue mould, caused by *Penicillium italicum* Wehmer, are among the most economically important post-harvest diseases of citrus world-wide.[1] Currently, measures employed to manage both diseases involve fungicides such as sodium *ortho*-phenyl phenate (SOPP), imazalil or thiabendazole, usually incorporated into waxes.[2] However, the use of fungicides is becoming increasingly restricted because of environmental and health concerns, as well as the development of resistance to these fungicides by fungal pathogens.[3,4] Therefore, interest in alternative methods of post-harvest decay control has been increasing.

Pre-storage heat treatments (curing treatments) appear to be a promising method to control post-harvest decay. Heat treatments can be applied to freshly harvested commodities by immersion in hot water, vapour heat, hot air or by a very short hot water drench over rotating brushes.[5–10] Besides physical methods, post-harvest heat treatments are currently practised, and control post-harvest diseases by direct inhibition of the pathogen and by stimulating certain host-defence responses. For instance, constitutive antifungal materials act as a first line of defence against invading pathogens. This is followed by the induction of several additional mechanisms, such as the building of a passive barrier to the pathogen by the production of lignin-like polymers catalyzed by phenyl ammonialyase (PAL),[11] synthesis of phytoalexins[12–14] and the biogenesis of several pathogen-related proteins such as chitinase.[15]

The first curing experiments on citrus fruit were performed in 1922 to reduce *Phytophthora citrophthora* Leonian infections, although, more recently, curing

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experiments have proliferated to reduce a dependence on agrochemicals.[16] The efficacy of curing as a protection against infection by various wound pathogens has been described for several citrus fruits,[10,17–19] although most of this research has focused on controlling *P digitatum*. Holding wounded and inoculated citrus fruit for 1–3 days at 33–36 °C and high relative humidity (90–96%) markedly suppressed green mould decay during storage.[17] Little has been published about the control of blue mould by curing. Recent studies carried out by Plaza *et al*[20] showed that curing 'Salustiana' oranges at 33 °C for 65 h controlled green and blue mould incidence on fruits stored at 20 °C for 1 week following treatment, but it did not effectively reduce blue mould on oranges stored at 4 °C for 2 months.

Carbonic acid salts, such as sodium carbonate and sodium bicarbonate, widely used in the food industry, are food additives allowed with no restrictions for many applications under European and North American regulations.[21,22] The antimicrobial activity of these chemicals has been described *in vitro*[23,24] and in a wide range of substrates as well. In 1928, Barger[25] showed that the immersion of citrus fruits in solutions of sodium bicarbonate reduced the incidence of green mould. Recent work showed that sodium carbonate solutions, used properly, approach the effectiveness of the common synthetic fungicides now in use to control green mould on lemons,[26] oranges[27,28] and mandarins.[29] Similar results were reported for blue mould on oranges and mandarins.[6,29]

The objectives of this study were to: (1) evaluate the efficacy of a curing treatment at 33 °C for 65 h against green and blue mould on oranges and lemons stored under ambient and cold-storage conditions, and (2) to explore the potential for improving this efficacy by combining curing with sodium carbonate on fruits stored under cold conditions.

2 MATERIALS AND METHODS

2.1 Fruit

'Valencia' oranges (*Citrus sinensis* [L] Osbeck) and 'Eureka' lemons (*Citrus limon* [L] NL Burm) were obtained soon after harvest from groves before any commercial post-harvest treatments were applied, and randomized. Oranges were grown in Baix Ebre–Montsià areas in Tarragona (Catalonia) and lemons were from the San Joaquin Valley (California).

2.2 Pathogen culture and inoculation methodology

Isolates used in the orange tests (conducted in Catalonia) were *P digitatum* isolate PDM-1 and *P italicum* isolate PIM-1 (both obtained from decayed citrus fruit in the Pathology Unit, UdL-IRTA Centre, Catalonia). Those used in the lemon tests (conducted in California) were *P digitatum* isolate M6R (obtained from JW Eckert, University of California, Riverside) and a strain of *P italicum* isolated from decayed oranges. All *Penicillium* spp isolates were cultured for 1–2 weeks on potato dextrose agar (PDA) at 25 °C. Spores were harvested by adding 9 ml of sterile, deionized water containing a drop of a wetting agent per litre to the Petri dish, rubbing the surface with a sterile glass rod, and passing the suspension through two layers of cheesecloth. The suspensions were adjusted to 1×10^6 conidia ml^{-1} with a haemocytometer. Fruits were inoculated by wounding each fruit once on the equator with a steel rod with 1-mm-wide and 2-mm-long tip previously immersed in the spore solution.

2.3 Control of green and blue mould by curing

A curing treatment at 33 °C for 65 h was evaluated on fruits artificially infected with *P digitatum* and *P italicum* as described in Section 2.2, and in naturally infected fruits. Control fruits were maintained at 20 °C for this period. After treatments, fruits were stored for 7 days under ambient conditions (20 °C and 90% RH) or under cold storage conditions of 2 months at 4 °C and 90% RH for oranges and 1 month at 10 °C and 90% RH for lemons. To simulate commercial shelf life, an additional 7 days at 20 °C and 90% RH was added to both orange and lemon storage periods. After storage, data were recorded as the percentage of decayed fruit.

Each treatment was applied to four replicates of 20 fruits each in tests with artificially infected fruits, or to four replicates of 60 fruits each with naturally infected fruits. The experiments were repeated twice.

2.4 Combination of curing and sodium carbonate

In order to simulate the different states of fruit injury and inoculation under commercial conditions, fruits were inoculated: (1) as described in Section 2.2 before each treatment (inoculated fruits), (2) after treatments with 15 µl of spore suspension in the wounds made before each treatment (injured fruits), or (3) fruits were inoculated before treatment as described in Section 2.2 and again after treatment with 15 µl of spore suspension (reinoculated fruits). *Penicillium italicum* was used in both orange and lemon tests. *P digitatum* was used only in lemon tests because green mould was satisfactorily controlled during post-harvest storage on oranges by curing alone. Naturally infected fruits were also treated.

The treatments applied were: (1) a curing treatment at 33 °C for 65 h, (2) a 2.5-min immersion in sodium carbonate solution (20 g litre^{-1}) followed by a brief rinse in tap water, and (3) the combination of both treatments where immersion in 20 g litre^{-1} sodium carbonate solution immediately followed a 65 h curing treatment at 33 °C. Among naturally infected fruits, an additional treatment of immersing fruit in ambient temperature water for 2.5 min was added. Control fruits were stored at the cold storage temperature after inoculation or, in the case of naturally infected fruits, placed directly into cold storage. Treated oranges were stored for 2 months at 4 °C and 90% RH and treated lemons were stored for 1 month at 10 °C and 90% RH. After cold storage, fruit were stored for 7 more days at 20 °C to simulate commercial shelf life, then the percentage of decayed fruit was recorded.

Each treatment was applied to four replicates of 20 fruits each in artificially infected fruits or to four replicates of 60 fruits each in naturally infected fruits.

2.5 *In vivo* inhibition of spores of *Penicillium digitatum* and *Penicillium italicum* in orange wounds

The inhibition of *P digitatum* and *P italicum* spores was studied on disinfected and wounded 'Valencia' oranges. Each fruit was wounded once with a steel rod with a 1-mm wide and 2-mm-long tip. Fruit were inoculated with 15 µl of a spore suspension adjusted to 1×10^6 conidia ml^{-1} 2 h before the curing treatment, immersed in water for 2.5 min, immersed in sodium carbonate solution (20 g $litre^{-1}$) for 2.5 min, or the combination of curing and sodium carbonate was applied. Control fruits were kept at 20 °C until spore viability was assessed. Five oranges constituted each replicate and a piece of peel surface (2.5 cm^2) that included the inoculated wound from each orange was removed with a cork borer (five pieces per replicate). The peel surface segments were shaken in 25 ml of sterile distilled water containing a drop of a wetting agent per liter on a rotatory shaker for 20 min at 150 rev min^{-1} and then sonicated for 10 min in an ultrasonic bath to improve detachment of spores from the tissue.

Serial tenfold dilutions of the washings were made in phosphate buffer (50 mM, pH 6.5) and plated on Dichloran Rose-Bengal Chloramphenicol Agar (Panreac Quimica SA, Spain). After 3 days at 25 °C, the colonies were counted and the number of viable spores per wound was calculated. There were four replicates per treatment.

2.6 Statistical analysis

Effects of treatments on the incidence of green and blue moulds were analyzed using analysis of variance applied to the arcsine of the square root of the proportion of infected fruits. This transformation was used to improved homogeneity of variances. The least significant difference (LSD) procedure was used to separate means. Statistical significance was judged at the $P \leq 0.05$ level. Paired t tests were applied in some tests.

3 RESULTS

3.1 Effect of curing treatment

Curing oranges and lemons at 33 °C for 65 h completely controlled green and blue mould incidence on oranges and lemons inoculated with *P digitatum* and *P italicum* and stored at 20 °C for 7 days (Fig 1).

When inoculated fruits were stored under cold storage conditions, the curing treatment also significantly reduced both green and blue mould on oranges and lemons; however, the magnitude of this reduction was not high (less than 80%), except for green mould on oranges (Fig 1).

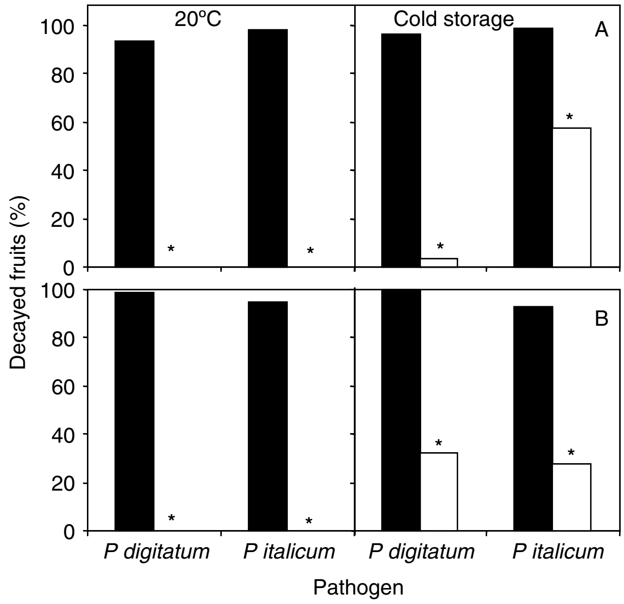

Figure 1. Influence of storage conditions on the incidence of decayed fruits on (A) 'Valencia' oranges and (B) 'Eureka' lemons inoculated with *Penicillium digitatum* or *Penicillium italicum* (□) followed by a curing treatment at 33 °C for 65 h or (■) untreated, after 7 days at 20 °C or 2 months at 4 °C on oranges and 1 month at 10 °C on lemons plus 7 days at 20 °C and 90% RH. Asterisks indicate treatments for each pathogen and storage condition that are significantly different ($P < 0.05$) according to the t-test.

On naturally infected oranges (Fig 2A), the incidence of green mould and blue mould was significantly reduced by the curing treatment both when the fruits were stored at 20 °C and at 4 °C following treatment. Curing naturally infected lemons at 33 °C for 65 h significantly reduced green mould incidence after each storage period (Fig 2B). Conversely, no significant differences were found in the incidence of blue mould when lemons were stored at 10 °C for 1 month plus 1 week at 20 °C, although the incidence of blue mould was very low among these fruit. Blue mould was not observed on lemons stored at 20 °C for 7 days.

No significant differences in the incidence of decay caused by other pathogens such as *Geotrichum candidum* Link were observed between cured and untreated fruits, although their incidence was low.

3.2 Combining curing and sodium carbonate on cold stored fruits

On lemons inoculated with *P digitatum* after the treatments were applied (injured fruits), efficacy was significantly improved when curing at 33 °C for 65 h was followed by immersion in sodium carbonate solution. The combination resulted in an 80.7% decay reduction (13.7% green mould incidence) compared with the curing treatment alone (Fig 3). When lemons were inoculated before the treatments (inoculated fruits), or both before and after the treatments

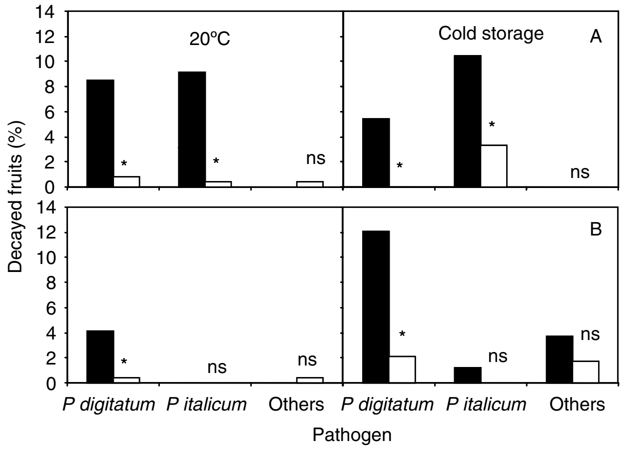

Figure 2. Influence of storage conditions on the incidence of decayed fruits on naturally infected (A) 'Valencia' oranges and (B) 'Eureka' lemons (□) treated with a curing treatment at 33 °C for 65 h or (■) untreated, after 7 days at 20 °C or 2 months at 4 °C on oranges and 1 month at 10 °C on lemons plus 7 days at 20 °C and 90% RH. Asterisks indicate treatments for each pathogen and storage condition that are significantly different ($P < 0.05$) according to the *t*-test (ns is not significant at $P < 0.05$).

(reinoculated fruits), no significant differences were found in green mould incidence among fruits cured at 33 °C for 65 h alone or followed by sodium carbonate treatment after storage for 1 month at 10 °C plus shelf life at 20 °C, although the percentage of decayed fruit was reduced more than 65% compared with untreated lemons (Fig 3).

On oranges stored at 4 °C for 2 months plus 7 days at 20 °C, the combination of curing followed by sodium carbonate treatment significantly improved the control of blue mould compared with each treatment alone (Fig 4A). This combined treatment improved control of blue mould by 61%, 59% and 28%, among injured, inoculated and reinoculated fruits, respectively, compared with the curing treatment alone.

A similar pattern was observed when treatments were applied to lemons (Fig 4B). The combination of curing followed by sodium carbonate treatment improved the efficacy of curing alone by 70%.

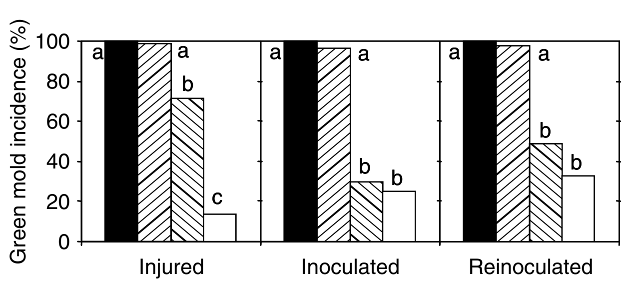

Figure 3. Incidence of green mould on injured, inoculated and re-inoculated 'Eureka' lemons, following (▨) treatment with sodium carbonate solution at 20 g litre^{-1} for 2.5 min, (▧) curing at 33 °C for 65 h, and (□) combination of curing and sodium carbonate solution, after 1 month at 10 °C plus 7 days at 20 °C and 90% RH. Control fruits (■) were kept at 10 °C. For each inoculation type, columns with the same letter are not significantly different ($P < 0.05$) according to the least significant difference test (LSD).

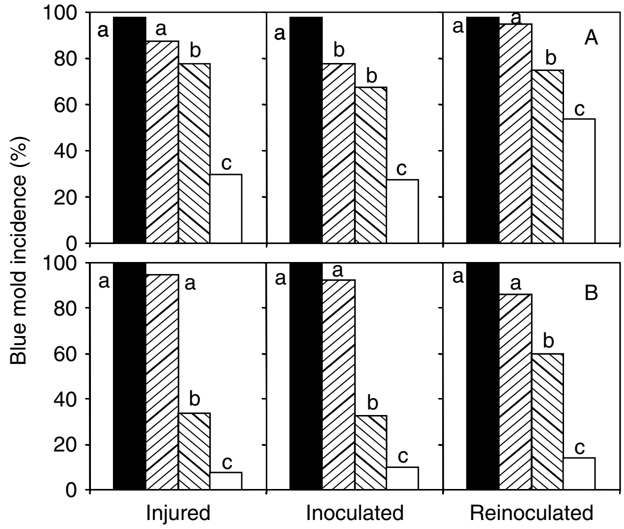

Figure 4. Incidence of green mould on injured, inoculated, and re-inoculated (A) 'Valencia' oranges and (B) 'Eureka' lemons, followed by (▨) treatment with sodium carbonate solution at 20 g litre^{-1} for 2.5 min, (▧) curing at 33 °C for 65 h, and (□) combination of curing and sodium carbonate solution, after 2 months at 4 °C or 1 month at 10 °C (orange and lemon tests, respectively) plus 7 days at 20 °C and 90% RH. Control fruits (■) were kept at the same cold studied temperature. For each inoculation type, columns with the same letter are not significantly different ($P < 0.05$) according to the least significant difference test (LSD).

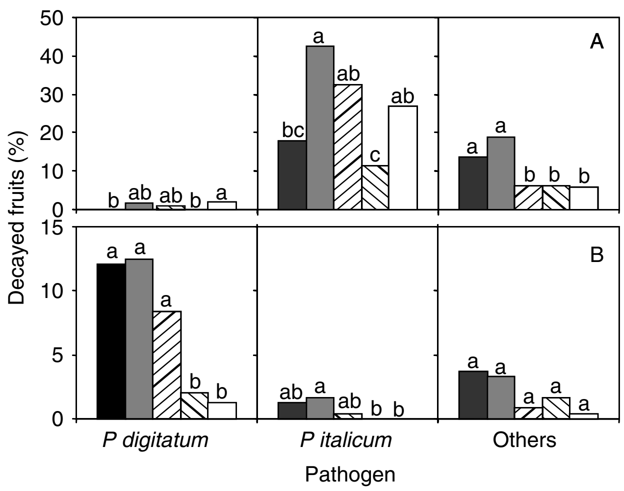

Figure 5. Incidence of decayed fruits by *Penicillium digitatum, Penicillium italicum* and other pathogens on naturally infected (A) 'Valencia' oranges and (B) 'Eureka' lemons (B) followed by treatment with (▩) water for 2.5 min, (▨) sodium carbonate solution at 20 g litre^{-1} for 2.5 min, (▧) curing at 33 °C for 65 h, and (□) combination of curing and sodium carbonate solution, after 2 months at 4 °C or 1 month at 10 °C (orange and lemon tests, respectively) plus 7 days at 20 °C and 90% RH. Control fruits (■) were kept at the same cold studied temperature. For each pathogen, columns with the same letter are not significantly different ($P < 0.05$) according to the least significant difference test (LSD).

On naturally infected oranges and lemons (Fig 5), green and blue mould incidences after each cold-storage period were not significantly reduced by the combination of curing followed by sodium carbonate treatment compared to curing alone. The same results were obtained on decay caused by pathogens other than *P digitatum* and *P italicum*.

Table 1. Viability of *Penicillium digitatum* and *Penicillium italicum* spores in wounds on 'Valencia' oranges dipped in water for 2.5 min, treated with sodium carbonate solution at 20 g litre^{-1} for 2.5 min, cured at 33 °C for 65 h, or the combination of both; control fruits were stored at 20 °C after inoculation[a]

Treatment	*P digitatum* spores per wound[b]	*P italicum* spores per wound[b]
Control	5.57×10^3 a	3.28×10^3 a
Water	3.28×10^3 a	2.06×10^3 ab
Sodium carbonate	1.03×10^3 b	5.75×10^2 b
Curing 33 °C/65 h	6.25×10^1 c	1.88×10^2 c
Curing 33 °C/65 h + sodium carbonate	<6.25 d	<6.25 d

[a] For each pathogen, treatments with the same letter are not statistically different ($P < 0.05$) according to the least significant difference test (LSD).
[b] Limit of detection: 6.25 spores per wound.

3.3 Viability of *Penicillium digitatum* and *Penicillium italicum* spores within wounds after treatments

When wounded oranges inoculated with *P digitatum* and *P italicum* were dipped in a sodium carbonate solution for 2.5 min, the proportion of viable spores of both fungi was reduced about 70% compared with fruits dipped in water for 2.5 min (Table 1). In contrast, curing at 33 °C for 65 h resulted in an 88.8% reduction in the viability of *P digitatum* spores and in a 94% reduction in the viability of *P italicum* spores compared with control fruits.

When the curing treatment was followed by sodium carbonate treatment, the reduction in spore viability of both fungi exceeded 90% compared with the curing treatment alone.

4 DISCUSSION AND CONCLUSIONS

Successful control of green and blue moulds on oranges and lemons was obtained by a curing treatment at 33 °C for 65 h when the fruits were stored at 20 °C. Similar results were obtained by Stange and Eckert[19] for green mould decay with similar temperature and curing conditions. They reported that curing lemons at 32 °C for 48 h resulted in *P digitatum* control comparable with dipping the fruit in a 1 g litre^{-1} solution of the fungicide imazalil. Lanza *et al*[18] demonstrated that curing for 3 days at 32 °C in a water-saturated atmosphere was very effective in reducing decay initiated from 24-h-old infections of *P digitatum* on lemons and Valencia oranges. When fruits were destined for long-term storage at low temperatures, effective control of blue mould on oranges and lemons and of green mould on lemons was not achieved by a curing treatment. Similar results had been obtained in previous work with Salustiana oranges.[20]

The mechanisms by which curing treatments reduce decay involve complex interactions with many lines of response such as mechanical barriers to micro-organisms, antimicrobial chemical compounds and pathogenesis-related (PR) proteins.[15,30] Direct effects of heat on fungal pathogens have also been reported by several workers. Heat inhibits or delays germ tube elongation or kills spores, thus effectively reducing inoculum size and subsequent lesion development.[15,18] Since the effectiveness of curing treatments depends principally on the host, this could explain the different decay control we obtained between oranges and lemons with green and blue moulds. It is probable that lemon and orange peels possess different capabilities of producing chemical or mechanical barriers to infection, antifungal compounds or lignin-like materials that subsequently influence the inhibitory effect observed on *P digitatum* and *P italicum* infections.

The level of effectiveness of sodium carbonate solution alone in our work was low because the concentration used was low compared with that reported in other research,[6] and we rinsed the fruit after sodium carbonate treatment, which also can reduce its efficacy. We minimized sodium carbonate efficacy in order to detect additive activity when it followed curing treatments.

Sodium carbonate solution significantly improved control of blue mould decay when it followed a curing treatment compared with this treatment alone on both oranges and lemons, and on injured, inoculated and reinoculated fruits. This broad spectrum of effectiveness, even controlling infections on fruit inoculated after the treatments were applied, is a clear advantage compared with other alternative control methods that possess primarily a curative effect. Sodium carbonate by itself is a poor eradicant and does not kill spores rapidly.[24] Similarly, we showed that the viability of spores of *P digitatum* and *P italicum* in wounds on fruit immediately after dipping in a sodium carbonate solution was reduced about 70%; therefore, substantial numbers of the spores remained germinable. Palou *et al*[6] also reported that *P italicum* spores from inoculated wounds on oranges treated with sodium carbonate or sodium bicarbonate solutions germinated and developed colonies, indicating that the pathogen survived the treatment. They observed an increase in decay incidence that occurred during the second-week storage period at 20 °C, which suggests viable spores were present. Therefore, the effect of carbonate treatment is primarily fungistatic, and it does not provide persistent protection of the fruit from re-infection. In contrast, defence mechanisms induced in the host by curing could complement sodium carbonate activity because they protect the fruit from re-infection. Moreover, it is important to note that curing is also considered a curative method *per se* due to the direct effects of heat on the pathogens. It is even more effective than carbonate immersion, as shown by a decrease in spore viability of more than 10-fold for *P italicum* and almost 100-fold for *P digitatum*. Other workers have focused on alternate control methods that have both preventative and eradicative effects. Effective control of green mould on oranges by following sodium carbonate or sodium bicarbonate treatments with applications of the biocontrol antagonist *Pseudomonas syringae* van Hall strain ESC10[28] or *Pantoea agglomerans* (Ewing & Fife) Gavini strain CPA-2 has been reported.[31,32] Biological control antagonists, which can persist on the fruit for long periods, may also provide this protection.[28]

When curing followed by sodium carbonate treatment was applied to naturally infected oranges and lemons in semi-commercial-scale tests, the results obtained with artificially inoculated fruits were not achieved. The combination treatment did not enhance the green and blue mould control provided by the curing treatment, and even resulted in a significantly higher percentage of green and blue mould infections in comparison with curing alone on oranges. These results can be explained because the response of pathogens to heat can be influenced by several factors, such as the moisture content of spores, age of inoculum and inoculum concentration.[33] However, it is important to note that, among naturally inoculated fruit, the variation in the incidence of green and blue moulds was high (coefficient of variations were 123.9% and 23.7% for orange tests and 34.3% and 120.9% for lemon tests, respectively).

The protection levels against citrus post-harvest diseases provided by synthetic fungicides are, in general, difficult to achieve with most of the alternative control methods that have been evaluated. Therefore, considerable research is currently focused on the combination of complementary physical, chemical and biological treatments. Combining curing at 33 °C for 65 h followed by immersion in a sodium carbonate solution resulted in an effective control of two important post-harvest diseases on citrus fruit, and even provided protection from re-infection. However, the variable results obtained in naturally infected fruits imply the need for further confirmatory research, because our inoculation methods may not have simulated the infections that occurred on these naturally inoculated fruit.

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